

Purcell, Robert 2005

Dr. Robert Purcell Oral History 2005

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Oral History Interview with Dr. Robert H. Purcell (NIAID, LID)

Conducted on December 7, 2005, by Dr. Lisa K. Walker

At the National Institutes of Health, Bethesda, Maryland

Abstract:

Dr. Purcell describes his education and training, his early career in the Laboratory of Infectious Diseases, and his work since he has headed LID studies of hepatitis viruses. He describes how the LID broadened its work, from a focus on viruses causing respiratory diseases through the early 1960s, to include hepatitis as well as gastroenteritis viruses. He also discusses internal collaborations at NIH and extramural collaborations, and many of the chance findings and coincidences that helped to further study of hepatitis and control of infection. Dr. Purcell also comments on how advances in technology and instrumentation have influenced the study of hepatitis viruses.

Lisa Walker: I would like to start out by hearing a little bit about your growing up, and your family and your decisions as you entered university and training in chemistry to begin with.

Robert Purcell:

Okay. I was born in Iowa and moved to Texas when I was six months old, and spent my first nine years in Dallas, Texas. Then my family moved to rural Oklahoma, and that's where I lived until I finished college and then went off to medical school. I guess I developed an interest in science from my brother, who was in college and was taking science courses, [and] who would come home with interesting little tidbits about things, and that really kind of excited me. The school I went to, since it was in rural Oklahoma, actually was a minimal school. It had very little science, no foreign languages, a poor math program. It was really not much of a school. So I went to a junior college, which was relatively close to where I lived in Oklahoma, for the first two years, and there I met a young Hispanic chemistry teacher, who was just very exciting – took a real interest in his students, and a number of us with a rural background and poor training really kind of blossomed in that setting. I had done reasonably well – I had done quite well, actually, in high school, but it wasn't a challenge. My mother was an English high school teacher, and my father was a science teacher in another school. So, I had an education background, but the environment was not really stimulating. In college, I got very interested in chemistry and decided to major in that and went on to a four-year college for my second two years. And I was, I think, [in] the last class of Oklahoma A & M, because – before it became Oklahoma State University – it gentrified.

[I] completed that, and then for reasons that aren't completely clear, I became interested in medicine. I think it was one of my advisors at junior college who had gone through my test scores and such and had asked me if I had ever thought about medicine. I said no, I really never thought anything about that – didn't have anybody in my family who had ever done that sort of thing. But then, the more I thought about it, the more I thought "Hm, it would be interesting." I decided by then I did want a scientific career, and I had that usual dilemma of whether to get a PhD degree or whether to get an MD degree, and so I wrestled with that for a while and finally decided that an MD degree would be what I wanted to do. So I applied to several medical schools and actually was accepted to all of them, but, being a Southerner at heart, I didn't consider any schools outside of the South. I started at Baylor Medical College in Houston, and the combined programs were just beginning then, so that school didn't have an MD/PhD program, but it had an MD/Masters degree program. So I got into that, and I took an extra year to get a Masters degree in biochemistry. And then I transferred from Baylor to Duke University, where I finished medical school and took an internship in Pediatrics.

The war in Vietnam was going on about then, so a very good option that came my way was to become an Officer in the Public Health Service as an Epidemic Intelligence Service Officer at CDC. So after my internship – I could have actually stayed out of the draft by [entering] the Berry Plan and completing a residency, but I really wanted to get more into the science aspect rather than clinical practice. I enjoyed the – as much as anybody enjoys medical school – I enjoyed the pediatric internship as much as anybody enjoys an internship, but decided I really did want to go into science.

So I got in the Epidemic Intelligence Service at CDC and was moved there for two months of standard training to be an EIS Officer. At the time, NIAID was flush with money from somewhere, so they had money to support several positions for vaccine development. They were big into vaccine development at the time, so [Dr. Robert M.] Bob Chanock, who was my lab chief for many, many years, came to Atlanta, and it was sort of like the slave market. He identified two of us in that class of EIS Officers to come to the NIH. So we both came to the NIH, and then we basically trained in the laboratory here, which was [in] building 7 at the time, for – it must have been part of the year, anyway. And the other fellow, Buck Edmonson [?], who came with me as an EIS Officer, was seconded to [the Marine Corps base in] Camp Lejeune, North Carolina, and I stayed in the laboratory here.

At the time, this was shortly after Bob Chanock's group had developed a new vaccine for adenovirus-based disease, which was rampant in military populations. And so this was a field trial to test the efficacy of that vaccine, the preliminary efficacy of which had been shown in volunteers here at the Clinical Center. So Buck was sent down to manage the field trial. To do that, he had to go down and set up at Parris Island, South Carolina, at the basic training facility for Marines, at the beginning part of the study, where the vaccination of new recruits coming in took place. And they were monitored through their training there, and then they were transferred to Camp Lejeune, North Carolina, for advanced training. And they were followed there, and that's where a lot of the disease actually normally occurred. So I went down for several months to Camp Lejeune to help with the field trial, and that was exciting, because it turned out to be a superb vaccine. It just wiped out adult acute respiratory disease, which is mainly caused by adenoviruses in the military facilities, so much so – and I'll just have to tell – it's an ironic story.

For years and years and years, [the adenovirus vaccine] was administered [by the FDA] as an IND [Investigational New Drug], because there wasn't really a market for it, so it was just used in [the military]. I think for 15 years maybe, the longest running IND in history. In recent years, several years ago, the higher echelons in the military, in their infinite wisdom, decided that there wasn't any respiratory disease in that population, so "why are we wasting our time with a vaccine?" So they didn't continue the vaccine. Of course now, they had epidemics of the same disease, have actually had several deaths, which had been a more common occurrence many years before the vaccine. So that "perfect storm," plus the fact that the company making the vaccine, Wyeth, didn't maintain the seed stock for the vaccine, led to a situation in which there was no vaccine, and no way to make it. Until seed stocks were reconstituted [and] safety tested, so that's what's going on now. The military has not had a vaccine because of ineptitude, actually.

LW: I had read that in one of the interviews we have transcripts of, [in which] Chanock has talked with people from our office, but I didn't know really where we were in 2005 on that. Okay, that's interesting.

RP: So that's how I got here, and that's how I got interested in respiratory viruses. Actually, when I was at Baylor doing my Masters degree work, I was in the Biochemistry Department, but I was seconded to the virology laboratory of [Dr. Joseph L.] Joe Melnick.

LW: Okay. This is what I wanted to ask you...

RP: That's how I met Joe Melnick, [and] he taught me my initial virology in his lab. And then when I transferred to Duke, I hooked up with Joe Beard, [Dr.] Joseph [W.] Beard, and Joe was a retrovirus guy. He did a lot of work on avian retroviruses and reverse transcriptase. Actually, as a product; it came out of those studies, although it was not seen as a reverse transcriptase at the time. It was seen as a viral enzyme, which they purified from virus obtained from hundreds of chickens that had been infected. I worked in that laboratory on avian myeloblastosis virus while I was there, and so learned more virology. So when I came here, I had a little bit of experience. And so I started on respiratory viruses but quickly got interested in mycoplasmas, because, as you know, from reading Bob Chanock's [interview transcript], he was one of those who discovered that primary atypical pneumonia was actually caused by a mycoplasma. So that was an active program in the lab when I was here.

And then I began looking at some of the other mycoplasmas that had been discovered or were being discovered, and we found that we were just scratching the surface, [that] there were a number of mycoplasmas yet to be discovered. So we discovered and described several of those, but it turns out that *Mycoplasma pneumoniae* is by far the most important of that group in terms of human disease. So, about that time – this is another story of Bob Chanock. When Bob took the job here [at LID], when he was offered the job by [Dr. Robert J.] Bob Huebner, before that, he said, "OK, I'll work on respiratory viruses," because he'd already discovered several of those. But he said, "I want to retain the right to work on other things if I want, when the time seems right." And hepatitis was one of those things he had been interested in working with. So after I was here a couple of years, I guess, that interest bit him, [and] he began looking for somebody to set up a hepatitis program. I was still a low man on the totem pole at that time, [and] he actually tried to strong-arm a number of the other people in the Laboratory to take on the hepatitis project. [Dr. Maurice A.] Maury Mufson, who was doing respiratory work at the time, reluctantly agreed to do that, but then he really didn't do anything with it. So not too long after that, Bob made the rounds again.

I always tell this story when there's a party for Bob or any sort of honor for him: the story of how I got into hepatitis. I just remember, one day, I had the lab at the end of the hall, which was the third floor, south, in building 7 at the time. And I heard him coming down the hall, going from office to office. Pretty soon I hear him stomping out of an office, into the next office, trying to get each person along the way to work on hepatitis. So, being the last person on the floor, and the lowest man on the totem pole, I got that job. So that's how I really got into hepatitis.

LW: There are some things that I want to ask you about your earlier training, but I'm curious, now that we're talking about this moment. I hadn't known that [hepatitis] was Dr. Chanock's interest to begin with. What was it, then, at that moment – what year was this, was this after 1965? Was this after the [identification of the] Australia antigen?

RP: It was about '63, '64; in that period of time. It was just in the same general time frame as [the discovery of] Australia antigen. We actually started before that. And I think the reason that Chanock wanted to start then, even though no one had made any progress on hepatitis, I mean, [was that] it was fairly clear at that time that there were probably two viruses: hepatitis A and hepatitis B. But little was known about either one. I think what made the decision for him was he had talked with someone – he used to go swimming a lot – you've probably heard...?

LW: I had seen reference to this.

RP: And I think one of the people he swam with was someone in the Clinical Center, or maybe the wife of someone in the Clinical Center, who knew about what was happening in the heart surgery program.[1] This was a new program; heart surgery was a brand new thing back then. They had set up a unit in the Clinical Center here to do open heart surgery, primarily for valve replacements and that sort of thing. And at the time, the technology was pretty primitive. To do it, it required an average of about 14 to 18 units of blood to do the surgery. And of course there were no tests for screening blood. It was known from earlier studies and volunteer studies that at least one kind of hepatitis was transmissible by blood, but no one really knew how many people were infected, and there was no way of determining that. So Bob wanted to set up a prospective study of heart surgery patients. We went to [Dr. Andrew Morrow]. We went to see to him, and he said, "Sure," [that] we could do that.

So we set up a program whereby we would get a blood sample from patients who would be undergoing surgery, to take [a sample] Monday morning, [as they] came in for the week's surgery, get a blood sample from them, and then – if they survived the surgery, [which not all of them did] – follow them at weekly intervals for the first couple of months, and then at monthly intervals for six months, and then perhaps indefinitely after that. Bob had an epidemiologic nurse, but either she or I or both of us would go over, or go out around about four states here, to the homes of these people – because they were generally too sick to come out. And [we would] bleed them and see how they were doing, so I saw a lot of the countryside doing that. And then, as that study was progressing – [Dr.] Harvey [J.] Alter, [who] at the time was down finishing his residency in hematology at – I think it was at George Washington University, George Washington or Georgetown, one or the other. He'd come back to NIH, and [Dr.] Paul [V.] Holland, who was the director of the Blood Bank about that time, had set up a hepatitis study also following patients. So we sort of combined our resources, and Harvey then took over the management of that study when he came up here, and so he's been running that study ever since.

What we found – we analyzed the data based on enzyme elevations – was that, of those patients, overall, about a third of the patients developed at least biochemical hepatitis. And if you look at those patients who were receiving principally or exclusively commercial blood, 50% of – can you believe that? – half of them developed hepatitis, transfusion-associated hepatitis.

So Harvey, you know, worked with [Dr. Baruch S.] Barry Blumberg, when he was here, when he was here *initially*. Harvey was one of the co-discoverers of Australia antigen. By then, Barry had gone off to Philadelphia, I think, and had [theorized a connection between] Australia antigen [and] several diseases. Eventually, he got it right, that it caused principally hepatitis, but at one point he thought it caused leukemia, Down syndrome, and a whole bunch of other things. At any rate, [Dr. Alfred M.] Fred Prince also clued into this, an association with hepatitis, and so both of them really kind of discovered that association. So Paul Holland set up the test in the Blood Bank and then began looking, and we developed some more sensitive tests for Australia antigen. And [what was] even more important, [these were] the most sensitive tests for antibody to it. And we found that really only a third of the transfusion-associated hepatitis patients had been infected with hepatitis B, and the other two thirds must have been infected with something else. The only other virus at that time that was recognized was hepatitis A, but there were no tests for hepatitis A. So – this was another one of these things coming together to be really exciting, and that was that, just before this, or at about this same time, [Dr. Albert Z.] Al Kipikian, who, you know, is one bay over [from me in the lab]; Al had gotten a little bit discouraged with his scientific career – and wanted to go to Johns Hopkins [University] to get a Masters degree in Public Health. Bob Chanock dissuaded him from doing that, but instead contacted [Dr.] June [D.] Almeida in England, who was very proficient at electron microscopy, and [was] sort of pushing the boundaries of electron microscopy as a technology. So Al went and spent six months with her, learned the technology, and then when he came back, began casting around for how to exploit it. Another interesting project in the Laboratory [at the time] was another group of as-yet undisclosed, unidentified viruses, and these were the gastroenteritis viruses. It was clear that a lot of gastroenteritis was caused by something for which there was no recognized etiology. So the group that Al worked with, that part of the Laboratory got some materials from an outbreak of epidemic gastroenteritis in Norwalk, Ohio, and they did volunteer studies with it. And they found that they could transmit that disease to volunteers, and they could characterize it, and they did that. A short incubation period, short duration, vomiting and diarrhea disease, different symptoms in different people; you recovered the agent, but you couldn't identify the agent. You just knew you had a transmissible agent. So, Al applied IEM [immune electron microscopy], the technique that June Almeida was working with – particularly, it was the modification of immune electron microscopy, in which you mix the virus with the antibody, and if you have one [of those two], you can look for the other. And so he took paired sera from the volunteers, and he took acute-phase stool samples from the volunteers, and he mixed them together and he looked at them in the microscope using this technique, and what he found was: the pre-serum in general didn't show anything, but the acute serum reacted with the virus particles and formed these "islands" of virus particles, which you could then see –

LW: I see; that's how it works.

RP: – and that's how you could identify that what you saw, those little particles there, were the virus. So, you now had a test for the virus and a test for the antibody. So, Al was the first to describe any gastroenteritis virus, and that turned out also to be the first calicivirus, a whole new family of viruses. And then they found other related viruses – not identical, but other related viruses – and other outbreaks of gastroenteritis. These occur principally in adults, as opposed to infants and children. I contributed one of those, because I was visiting friends in Paris, had dinner at their apartment, just before I came back to the United States, and developed a severe diarrhea – vomiting and diarrhea – about 48 hours later. So, we were all in there collecting specimens then, so I collected a stool sample for Al, and he found calici-like virus in that, which turned out to be similar to one of the others they had. That's the, if you ever see the Montgomery-County agent, that was my contribution.

LW: Okay, so that's your personal contribution.

RP: [Dr. Stephen M.] Steve Feinstone was in the Lab as a postdoc with my group then, and we were casting about for something that we could do that would be interesting, because we now had a test for hepatitis A, but we didn't – I mean, [we had a test] for B, that's the Australia Antigen test, a test for antibody – but we didn't have anything for A, so we wanted to look for that. So, we cast about for who had good samples. [Dr.] Leon Rosen used to be in the Laboratory, and Leon – small world: I ended up in Leon's old office in building 7, and he left the Lab, actually before I arrived, and [he] did work in the South Pacific as an epidemiologist and virologist, very good [work]. He married this beautiful French-Polynesian woman from the Marquesas Islands, who was half Marquesan and half French. And then he was on the faculty at the University of Hawaii in Honolulu, and did studies all over the South Pacific of various diseases, among them, hepatitis epidemics. So, he sent us collections that he had generated. We didn't find hepatitis A virus in those. The ones we eventually did [find] came from [Dr.] Saul Krugman and his studies at the Willowbrook State School, in Staten Island, New York. [Krugman is] someone who's been really badly vilified, I think, completely unreasonably [for his role in the Willowbrook studies]. It wasn't his direct specimens [that contributed to our work], but a study that began with his specimens – from the children at Willowbrook, where both hepatitis A and B were endemic – [specimens which] he had given to the U.S. Army. They used one of these to inoculate, to challenge, prisoners in a prison study at the Joliet Correctional Center, or prison, in Illinois. And they did get sick; they developed hepatitis.

Those materials had been collected by the Army, and Dr. Marc [sp] Conrad, who was at Walter Reed [Army Medical Center] at the time, supplied [us with] paired samples from those prisoners, under code. By then Steve [Feinstone] had learned the [IEM] procedure from Al Kapikian, [and] he and Al would spend – and all three of us, sometimes – would spend hours in the electron microscope room until the wee hours of the night, talking baseball, because that was Al's big thing. And I think it was Steve, one night, who came across particles that looked like virus-like particles in the stool of one of the Joliet prisoners, with serum, I think, from the prisoners. At any rate, that was the first key, and then that was developed with other samples, other paired sera. We broke the code with the Army, and it broke exactly right, it was apparent that that's what it was. But a part of that study – I think we, how many samples did we have? It wasn't very many, but it's the kind of perfect epidemiologic study where, if you very carefully pick your reagents, pick your materials, you don't need ten thousand people to get a complete, 100% answer; you [simply] know. And that was the way it was with that study. It was clear that these particles were hepatitis A, never seen before, and we could detect the antibody to it.

So, we published that in *Science*.^[2] Then, the next thing we did was – by then, we'd analyzed with Harvey Alter and Paul Holland the heart surgery study patients, the ongoing study there. So we decided to see, now that we had a test for antibody of hepatitis A, [whether] any of these patients who developed hepatitis who didn't have hepatitis B, [whether] they had serologic evidence of hepatitis A. We looked at about 22 serum pairs under code from those patients, and not a single one did. Some of them had antibody to hepatitis A, because they had had it previously. And [later,] once other sensitive tests were developed, we were able to show that, overall, about 40% of the population had antibody to hepatitis A, and this was dependent upon age: older populations had more antibody, younger populations had less antibody. But this was the first evidence for a third human hepatitis virus, and we coined [that] as "non-A non-B" hepatitis virus. Then we worked here and with Harvey and with others trying to identify and characterize the agent. With moderate success: about four [separate research] groups simultaneously reported transmitting it to chimpanzees, and that was big, because that then allowed you to begin the biological characterization. We had, with [Dr. James] Jim Maynard at the CDC field station in Phoenix, Arizona, been the first to transmit hepatitis B to chimpanzees, and we'd reported that previously. So that's one of the reasons we all looked at hepatitis A and chimpanzees.

Those were some of the kind of important milestones of that time. Another one of these things coming together was that, about the time I was sort of being encouraged to come here, being enlisted to come here, Bob Huebner, with an interest in cancer viruses and leukemia viruses at the time, was casting about for a way to find them and identify them. He had teamed up with someone at the Oak Ridge National Laboratory who was an expert in ultracentrifuges. And I just saw him, just the other day, and I'll tell you the rest of that story. His name is [Dr.] Norman [G.] Anderson, and [he] helped develop and then modify the technology for separating U235 from U238. There are two primary ways of doing it: one is a gaseous diffusion method, but the other is a method that uses a gas centrifuge. You pass a mixture of – you convert uranium into a gas, uranium hexafluoride, which is a gas, and you put this through a very high-speed centrifuge, continuous flow centrifuge. And, because of the slight difference in the weight of U235 and U238, the U238 moves toward the edge of the centrifuge, because of the centripetal force, very high – pulls it, so you get a relative enrichment in U235 at the core of the centrifuge. Well, Norm Anderson got interested in things like viruses, partly because of Bob Huebner's discussions with him. Basically, he's the father of the modern ultracentrifuge, the centrifuges that the various companies now make, that are vital for separating out viruses. [Anderson] developed the concept of what he called "the virus window": he looked at the density and, basically, the size of about 55 different viruses, and he plotted these out on an X/Y axis, and he found that these viruses have unique characteristics: their buoyant density – that is, the density at which they float in some medium, like sucrose or one of these things – and the rate at which they can move through a substance, which is a combination of their size and their density. And he found that most viruses will end up in a certain place in a combination of great zonal separations and gradient density centrifugations. And he called this "the virus window": this is where you would find viruses. Bigger things, heavier things, lighter things, smaller things go elsewhere, but there's this place where you look for them, so this was a clever concept. One of the people he trained was [Dr.] John [L.] Gerin. John was at the Oak Ridge National Laboratory and had trained under Norm Anderson. So, Bob Huebner said it was too difficult to get people to take their virus preps.

LW: Okay.

RP: Huebner wanted a satellite facility locally here, close to NIH, where these kinds of studies could be done. So, they agreed to do that. Money came up for it somewhere – our institute, I think – and Norm suggested that John Gerin set that lab up here. It was really [intended] for leukemia viruses and cancer viruses and that sort of thing, but while the lab was being built, Bob Chanock invited John Gerin to come work in *this* laboratory. John and I worked together, and [at] about that time, another mutual friend – an old friend of Bob Chanock's, with whom I think he was in Japan – came down and gave a talk about hepatitis. He'd done some of the early studies with volunteers and such on hepatitis A, and he was a very nice guy. And that sort of piqued my curiosity and John Gerin's curiosity, so they got us thinking more about some of the things that we could do.

LW: But this was before Dr. Chanock had sort of forcefully recommended that you, as the low man on the totem pole, pick it up?

RP: It was sort of all in the same time frame, because we were collecting samples from the heart surgery –

LW: Okay, so the heart surgery [study] had already begun.

RP: He really wasn't doing anything with them, because we didn't know what to do with them.

LW: I see; it didn't have direction yet.

RP: So we thought, "Well, okay, the best thing we can do is collect the clinical materials that we'll need – the stuff to work with, if something comes along." We really weren't doing anything [except] going out and bleeding patients. So, John had the expertise in ultracentrifuges, and about this time Barry Blumberg reported on Australia antigen. Another strange coincidence was – and I'm pretty sure it was Norm Anderson who did this... In his studies, or maybe [in] his studies with Bob Huebner early on, [while] looking for leukemia viruses, they found virus-like particles in the serum, or the blood, of a child with leukemia, and they tried to do things with it. They couldn't isolate the virus; they couldn't do very much with it. But they did take electron micrographs of it. They saw it in the electron microscope. If you take those pictures and look at what Barry Blumberg described for Australia antigen and the hepatitis B virus, they were the same thing. And that was before Barry had described Australia Antigen. They just didn't know what to do with [what they had observed].

At any rate, by then John's lab was being set up, so we began to say, "Well, one thing we could do..." [one thing] John had expertise at, was to characterize Australia antigen. About this time Saul Krugman had done the first experiment that suggested that you could indeed make a vaccine against hepatitis B. He had done studies at the Willowbrook – what he was vilified for was for infecting mentally retarded children at the Willowbrook State School with hepatitis A or hepatitis B, or both. His justification – and in that time frame, I think it was a valid justification – was that all of these children got it. And he felt that it was important and ethical to study it in a controlled situation where you could at least learn something from it. Because they had no way of preventing it, because they didn't know what it was doing. So he began by showing that, indeed, it was transmissible; that you could transmit by mouth – one of the viruses, but not so well the other. That you could transmit both of them by inoculation; that you could demonstrate, by inoculating other children, what the period of infectivity was. You could determine the biochemical parameters of the disease from the very beginning, which you hadn't been able to do before. And, to his credit, he did – he talked with the parents, he got permission from the parents, he wouldn't do the study if they didn't provide – you could never do it today, because ethics have changed; at least the perception of ethics has changed. But I was a good friend of Saul's, and I've always defended what he did, because I knew him very well, and he was a very kind, wonderful physician, and he was very deeply hurt by [the criticism he received], because he really didn't understand the tremendous vituperation that was dumped on him.

At any rate, we got materials from him to begin – because he had collected these materials – to begin characterizing Australia antigen in terms of its virus window, how it centrifuged in terms of its density, how it centrifuged in terms of its zonal separation from other serum plasma components. And John showed that he could purify to a high degree of purity. Now, Saul, at the meantime, [in] some of the other studies he was doing, was thinking about making [a vaccine] – or he was trying actually to see if he could inactivate the viruses. One of the things he tried was heating serum from a patient with Australian antigen, with hepatitis B at least, I'm not sure of the timing... So, he heated it and then inoculated other children with that, to show that it was no longer infectious. Then he challenged those children to show that they were susceptible, and they were protected. So this was the first evidence for a crude, but somewhat effective, hepatitis B vaccine. So John and I picked up on that, and we thought, "Well, you have to do better than that. Let's characterize the antigen, see if it is the virus, if we can determine that or if it can be somehow inactivated in a more reasonable way to serve as a vaccine." And so John did those separations – they're beautiful separations – showed that he could highly purify it.

Now, Barry Blumberg was doing this also at the same time, but he never published it, so we never knew about it until subsequently. And then John expanded [on] that [work] to develop techniques for large-scale purification. We made a vaccine from a unit of plasma that Saul Krugman had collected from one of the children at Willowbrook, and we – by then, we were able to get a hold of some chimpanzees, and we had already shown that they were susceptible in the studies with Jim Maynard at the CDC. So we immunized chimps with that material: we inactivated it with formalin, [using] standard procedures for vaccine inactivation, and then we precipitated it with alum, and then we immunized chimps with it, and then we challenged them, and they were completely protected. It was very exciting.

LW: Yes

RP: It did not produce disease. We showed that it was inactivated, and then we showed that it generated protection, and we showed that this was associated with – probably associated with – antibody, which ultimately it was shown to be. So that was really the first demonstration of a practical hepatitis B vaccine. That was made from the plasma of this child, and the first licensed hepatitis B vaccine, made by Merck, was made from the plasma of individuals. So it was for many years – and in some developing countries, still is – made from plasma of chronically infected patients. It was a very effective vaccine.

At about that time, AIDS came on the scene. It was in 1980-'81, and it was right after the licensing of plasma-derived hepatitis B vaccine, which was in, I think, 1981. It took several years to figure out what AIDS was. It turns out that the purification procedure that – at least that Merck used; well, ours too, because we never could transmit anything else from it – completely removed or inactivated HIV and other viruses, too. There were never any other cases that I know of with problems. But, because much of the plasma that Merck used to make this vaccine came from young males from New York City, who subsequently were shown to be at high risk of hepatitis B or [HIV via their homosexuality], there was a great move to shift to another kind of vaccine. At about that time, molecular biology had reached the point where the gene that encoded the envelope protein of hepatitis B, which is what Australia Antigen is, could be cloned and sequenced and expressed in other systems. And John and I were just prepared to do that: we had hired someone expressly – he was a good molecular biologist, he's still at NIH, elsewhere at NIH – to express hepatitis B surface antigen in another system.

LW: So that you wouldn't have to use plasma...

RP: Yeah. But the rules and regulations for this in the United States were – they wouldn't allow you to do it. Some group, I think, went to Mexico to do it, but we couldn't do it. We had everything ready to go to do it, and I think it was eventually done either in Mexico or European someplace else.

LW: Because of caution about recombinant DNA techniques? I see.

RP: Yes, we just couldn't do the experiment. But then others did, and we moved to a recombinant vaccine.

LW: So that was the next step in the vaccines, from the inactivated one that you first did, and was then licensed?

RP: Yes. And one that's been used worldwide is expressed in yeast. Yeast is eukaryotic cell. And it was put in yeast, because the glycosylation of the hepatitis B surface antigen proteins – glycoproteins – is fairly complex, and the thought was that it might be necessary to have these glycosylated proteins, which you only get in eukaryotic cells, and yeast is a eukaryotic cell. It turns out that the expression of hepatitis B surface antigen in yeast does not involve glycosylation; it's not glycosylated at all. [The use of yeast] turned out not to be necessary, but [this process] does spontaneously form particles that look very much like the HBsAg particles that you see in the plasma of infected people. And it works very well, so that's the vaccine that's been used ever since.

LW: I was going to return to ask you more about your training, but now that we're talking about the vaccines – I'm curious whether there's any connection between [on the one hand] the work that you started out [performing], when you first were recruited by Dr. Chanock to do the vaccine evaluation studies for the adenovirus and on the other hand this later work on hepatitis B vaccines – is that important at all? Do you see that work as important at all, leading up to or contributing to what you eventually did involving hepatitis?

RP: Well, no, it was what I was assigned to do as a Public Health Service Officer. And actually, I stayed in the Public Health Service for 33 years, and then they finally threw me out, because they really don't like to keep people beyond 30 [years]. So, I'm now in a Title 42 position here. I was in the Senior Biomedical Research Service for a while, and then they transferred everybody to a Title 42 position.

It was important to learn those things, but my real interest was at the bench at the time. And so I really didn't want to do the field trials. I did a few others during that time, when Maury Mufson was here. I helped him with clinical studies of mycoplasmas and some respiratory viruses, [which] at that time we were doing at the District of Columbia Lorton Correctional Complex, or prison. And they had big studies at [the DC welfare nursery at] Junior Village, and [at] Children's Hospital, but I wasn't involved in those. But I really was more interested in more basic science, and so that's what I gravitated to, and that's what I ended up doing.

I like translational research. I like that space between the basic observations, which I like to make also, but then I like to go to the next step, and then I like to see the public health application. And I tell many people, "If you're here long enough, you have the tremendous pleasure of thinking of a new idea, looking at it at the bench, and then reaching the point where you see it as a public health measure." And that's true with hepatitis B vaccine. [It's] true for hepatitis A vaccine, also: we were involved with GlaxoSmithKline in developing the hepatitis A vaccine, which was [the] first licensed hepatitis A vaccine, the one they market worldwide now. We sort of got into that – you know, there's a lot of serendipity in science – and we were interested in vaccines, because that's what this Laboratory does. We were interested in trying to make a live attenuated hepatitis A vaccine, because that's what this Laboratory does, by and large. So we spent a good bit of time – the original isolation of hepatitis A virus was by Merck. It was [Dr.] Maurice Hilleman's group [that] had done that. [Dr. Philip J.] Phil Provost was the first to report that.

And then we cast about to find good samples, because our initial attempts were not successful. But a mutual friend from Australia, [Dr.] Ian Gust – whom we had known for many years, because he has an interest in epidemiology and infectious diseases – wanted to come and spend a sabbatical here with Steve Feinstone and me. And he brought along clinical materials that had been collected in Australia, and among these, was [one] from an outbreak – I think it was a mussel-associated outbreak of hepatitis A in Melbourne. And one of the samples was from a patient whose designation was HN175, and we began looking at those. And it turned out [that] this hepatitis A virus in this patient was among the easier ones to isolate in cell culture. So, we tried that one, and we had success fairly early on. We eventually went back and looked at some of the others we had tried without much success, and they turned out to be *the* most difficult to isolate. They were the last to isolate; it took years to isolate those.

At any rate, this virus then grew relatively well, and so we wanted to make a live attenuated vaccine. So we passed it in cell culture, and it did adapt. It grew better. We did studies in tamarins and in chimpanzees, so we could show that the cell culture-adapted virus actually was attenuated – didn't produce disease, but did produce immunity. That was really what we were looking for. In the meantime, we had teamed up with GlaxoSmithKline, who was interested in the hepatitis A vaccine, to do some collaborative studies to try to make and to evaluate the live attenuated vaccine. In the meantime, their primary interest was in an inactivated virus for hepatitis A.

They had set up a collaboration with another mutual friend from Switzerland who had an isolate that they were going to use as the vaccine strain for their vaccine. I suggested, "Why don't you look at this HN175 strain, just to see how it does for you." And it turned out it gave a better yield, it grew better, and so they dumped the other strain and went to HN175 and used that as the basis for their inactivated vaccine. We continued to look at a live attenuated vaccine, but what we found was what you often find with RNA viruses: the virus had a frightening ability to back-mutate or to pick up compensatory mutations. And sometimes, it would hang around in the host, in the tamarin or the chimpanzee, for months, and then emerge as a virulent virus again. So we finally decided it probably didn't have a future. But [by] then they had an inactivated vaccine that was going very well.

LW: Okay, so I'm beginning to understand more about [the approach to developing a] live attenuated [vaccine]. I wanted to ask you, for instance, for [hepatitis] B, first you sort of stemmed off of Krugman's work and developed that inactivated [vaccine], and was there no hope at that point, either to come up with a live attenuated vaccine for hepatitis B, or because you had the inactivated and it worked –

RP: No, the problem is [with] any virus that has a propensity to go under chronicity. Then you have to worry whether, maybe it's attenuated, but it may still cause a lifetime infection. Hepatitis B could lead to liver cancer, or all sorts of things.

LW: Okay, I understand now.

RP: It's certainly – it's not like HIV, it doesn't do that every time. And in fact, in adults, only about 5 to 7% of infections with hepatitis B virus go under chronicity, but that's high enough.

LW: It's high enough that you don't want to risk it.

RP: Yes, and in infants, it's about 90%. So, in an age group where you'd like to vaccinate them, it would be risky. That's the main reason.

LW: Okay. So it's chronicity and then it's also this issue of –

RP: Basically.

LW: But then another safety issue is the RNA viruses: their ability, the ease with which they revert to virulence. Okay.

RP: See, the thing about hepatitis viruses as a group, is: they don't cause cytopathogenic effect. Probably none of them actually cause a cytopathogenicity, and the disease, at least where it's been carefully looked at, is an immunological disease. So, making an attenuated vaccine leaves you pretty much with making one that simply doesn't grow as well or to as high a titer, so that the immune response is likely also not to be as good. You can't take out a gene, for instance, or modify a gene that's the cause of the pathology, because the virus, in the absence of an immune response, is pretty benign. And it keeps growing. So, it makes it difficult with those viruses. So, three of the five viruses go under chronicity and all of them are probably immunologically mediated diseases. So, an inactivated or recombinant vaccine probably makes more sense.

LW: Right. That starts to make sense [to me]. I keep hearing about the strategy at the Laboratory of Infectious Diseases being centered on live attenuated vaccines. But when you have [a vaccine] that works, and it works best [given the nature of the disease], then you go with that. I mean, you're not going to sacrifice something just because of this idea...

RP: No. Bob Chanock, as you know, trained under [Dr.] Albert [B.] Sabin, and Albert Sabin was sort of the father of live attenuated vaccines. And so Bob has been [oriented] very strongly in that direction.

LW: So that really does come from Sabin?

RP: Yeah, it does.

LW: Okay. I mean in terms of the Lab, that's the genealogy of it?

RP: And so many of the vaccines that have come out here [at LID] are live attenuated. The adenovirus – interestingly, the adenovirus vaccine was not attenuated. That was an ingenious thing: its success comes solely from the route of administration. Adenoviruses can infect the respiratory tract, where they cause respiratory disease, sometimes severe, and they can infect the gastrointestinal tract, where usually they don't cause any disease. A few strains are said to cause diarrhea, but those that principally cause a respiratory disease can infect that [gastrointestinal] tract without disease. So what they hypothesized was, "Gee, if we can bypass the respiratory tract, maybe it would work." So, they [prepared] the virus, and they dried it down and put it into gelatin capsules, and then they coated them with materials that converted them into an enteric capsule, where [it didn't] dissolve until [it entered] the enteric tract. And they administered those, and it was great. [The vaccine strain in the enteric capsule] infected; [it] caused a very good antibody response. [It] did not cause respiratory disease, and it wasn't transmissible to other people by that route. So, that was the basis of the vaccine. It's a wild-type, virulent virus that's used in that vaccine.

LW: But it's just kind of lucky that you have a virus of that nature, that you can do it that way.

RP: And it didn't work for a number of the other vaccines. There were several adenoviruses that were important to pediatric diseases: the lower-numbered [types] principally. But it just didn't work for them. It works for the military strains, which are 7, 14, and some outbreaks for 21 – those are the three important ones. And I think it works for all three of those; I know it does for 7 and 14. So, that was that approach. And then [Dr.] Brian [R.] Murphy, who has a very large program based entirely on live attenuated vaccines – [for] flaviviruses, for pneumoviruses, for metapneumoviruses, for parainfluenza viruses – contributed a lot to live attenuated vaccine for influenza. The initial work on cold adaptation was done by Dr. Hunein F. "John" Maassab in Michigan, Minnesota, or Wisconsin, one of those states[3], and he had patented all of that, did all of that [work on cold-adapted influenza A virus], but he never got it to the point of really being practical. Brian spent a lot of time working on that, introducing mutations and such, and what he did really served as the basis for MedImmune's FluMist vaccine that was just licensed a couple of years ago. Now, Maassab did his work 20 or 30 years ago. This gives you some idea [of] how long it takes sometimes to get from the bench to actual practical application.

LW: Right. I didn't realize that. And the surprising thing with hepatitis, it seems [to me], [from] reading those [historical] reviews [about discoveries and progress in hepatitis virus research] and just thinking about it, the surprising thing is the *rapidity* with which the knowledge has progressed.

RP: Pretty quick – there are some vaccines that have been quicker. I have a figure that I've shown at some talks in the past that [illustrates] what the interval has been between what I call an "enabling event" – which is either the isolation of a virus in cell culture where you can do something with it, or for those that don't grow in cell culture, the cloning and sequencing of the genome so that you can express the proteins – and the licensing of vaccine. I think the shortest probably was polio. That was just seven years. The others run about – most of them run nine, ten, eleven years, twelve years. The hepatitis viruses, I don't remember the exact period of time but it was in the ten- to fifteen-year range.

LW: Okay, yeah, so that's not so short [after all].

RP: That's not bad, really, to be able to move something that fast. And you can see also why it costs so much to make a vaccine. There's a lot of work that needs to be done. I'm not sure what the estimate is now. It's at least [\$] 200 million, maybe [\$]500 million to make a vaccine.

LW: To come up with a single product.

RP: To get through licensing. So it costs a lot to do that.

LW: I guess the other thing I'm thinking about in terms of hepatitis is [the importance of animal models. It] looks like it must have been a real obstacle that you didn't have an animal model, and that [indicates] why Saul Krugman's work at the Willowbrook was so important.

RP: Yes, and no animal model – people forget that. It was largely because of an increasing backlash about studies in volunteers that we began looking hard at animal models, and tested the chimp. That's where we got into that business. And I've been working with chimps now since 1965, '66, '67 – before 1970, anyway. And we're still doing that. And now there's the same hue and cry about doing studies in chimpanzees that there was [about studies] in children 30 years ago. It's increasingly difficult to do studies in chimps because of animal rights issues.

LW: And is this the kind of thing that I see, [for instance], when I look at [some of the current research at] the FDA, and they're coming up with different safety tests? This is not chimps, but it's with monkeys – safety tests including alternative neurovirulence screening tests such as mutant analysis by polymerase chain reaction and restriction enzyme cleavage, or MAPREC, devised by FDA researchers. Is that the same kind of issue driving this kind of research?

RP: Well, the FDA – their primary issue is safety, so they ask for what they feel is necessary for safety, but they feel the pinch from animal rights groups also. For many of these things, they want two animal models, if you can do it. If you don't – if it's something that is potentially really dangerous, they'd like two animal models. They don't require the chimpanzee; they recommend, they suggest the chimpanzee in some specific kinds of studies now that are being done more and more, and these are evaluations of human or humanized monoclonal antibodies to one thing or another. Usually, it's to some human protein, something like gamma interferon or something of that sort. And these are tremendously potent therapies now, but the problem is that these are directed against human components. You know, you want to turn off some cytokine system or something of that sort. Or there are ones that are directed against T-cell receptors or B-cell receptors, so that you can wipe out those cells if they are involved in the pathogenesis of the disease, and in some cases they are. But, [for that], the FDA recommends that the study be done in chimpanzees before it's done in humans, or before very much is done in humans. And the reason for that is: these very potent monoclonal antibodies that can destroy all the T-cells somebody's body carry a very great risk. In fact, I know of one instance in which one monoclonal antibody, which I think was directed against T-cell receptor, was tested in chimps in escalating doses. And they killed four chimps, because they completely wiped out the T-cells.

We had used that monoclonal antibody in chimps, but never to that level. And, so, that then was a reality that you had to be very careful in patients with these things. They can be lethal. So, that's one of the things that there's a very strong push to use chimps for.

LW: Well, we've talked so much now about your work at NIH. Part of what I had wanted to do was ask you more towards the beginning of your training –

RP: We'll come back, because there are some other really interesting things I want to tell you.

LW: Okay. Well, you mentioned that you worked in Melnick's lab, for instance. I was just wondering about some of the inspiration and maybe the puzzles that were inspiring to you in that beginning [time]. So, you were studying, for instance, chemistry, biochemistry, but you had this temporary time in Melnick's lab. What were you working on and what exactly was catching your interest at that time?

RP: Well, there was a virus that was described, actually by two people here at NIH, at about the time that I was starting my Masters degree in the Biochemistry department [at Baylor University]. [Dr.] A. Clark Griffin was my advisor there, and he was interested in the biochemistry of viruses, which was a developing field at the time. He was sent this paper by – [about] the SE [Stewart-Eddy] polyoma virus – Bernice Eddy and, I forget who the other one –

LW: Sarah Stewart, right?

RP: Sarah Stewart, yes; Sarah Stewart and Bernice Eddy. Because it really looked interesting; it was associated with multiple kinds of tumors in mice. It was reproducing; it was a cancer virus that grew well and did all kinds of interesting things. And the first blush of this was, "Gee, this is the cause of cancer. [It] causes all kinds of cancers. Here you have a model system: great!"

So, [Griffin] was interested in characterizing the virus. He wanted me to work on methods of purifying the virus, but I didn't know anything about virology at the time, so that's why he sent me to work with Joe Melnick, just to learn virology. And so that's what I did, and I just sort of followed around people in Joe's lab, and learned the general techniques of passing viruses and harvesting them, and looking for what they did in cells, keeping the cells sterile, and all those kinds of things. So that's really what I did with Joe; I didn't have a specific project with him. Although I did part of the work toward my Masters degree in that lab, because it really required virology [techniques].

And then I worked on ways to try to purify the [SE polyoma] virus: various kinds of column technology were becoming popular then – separations with chromatographic columns, various kinds of gels and media. So, I worked on that, and – I can't remember how we monitored the virus, I think it was by hemagglutination? It was a marker, but – I think it must have been hemagglutination. You could monitor where it was, coming through a column, what fractions it was in and that sort of thing. So, that was really the basis of my Masters thesis.

LW: Okay, and that was with polyoma, and then you moved – then you went to medical school...

LW: This is tape 2 with Dr. Robert Purcell. Again, I'm wondering about [your] inspiration. It sounds to me [as though], although you had the inspiration to go and get a medical degree, and that was what you decided to do rather than continuing with chemistry, and you did the pediatrics internship – was it really just a means to an end or... What was your driving interest at that point in what you were studying?

RP: Well, I didn't have a great compulsion to get a residency, because I was interested in the basic science, but from the perspective of biology. That actually has been my driving force from the very beginning, and that's why I got an M.D. degree instead of a Ph.D. degree, because I really was interested in the context of biology. And I think it was a very good idea; I would do it again. If I could do it again, and they had them at the time, I would do an MD/PhD program. That would be the best of both worlds. But a medical program is a fairly stringent learning exercise. You learn a lot, and the lab work I did, the Masters degree, taught me how to do experiments, how to think through problems, and that sort of thing. So that was really, I think, what drove me.

LW: So it was still the bench science that held the greatest interest for you, I see. I'm still curious to know [about] – but I'm wondering if I need to talk to someone else about that – [the] Vaccine Development Branch and how that worked. I mean, this sounds like something I've read about a little bit with other labs, like [Dr.] Edwin Lennette's lab in Berkeley, California. This was basically how NIAID [conducted] evaluation of candidate vaccines?

RP: It was a way – I mean, some work was just done here by the people who were here and who came into this lab directly to NIH, rather than to another mechanism, but Bob was expanding these programs at the time, and the EIS program populated most of the state public health laboratories around the country. That's how Ed Lennette, I think, got involved. One of the things they did was [to] second EIS Officers to various health departments in various states, and they were the ones who would go out in a given state to do an epidemic evaluation, and then they'd do that sort of in conjunction with CDC, but they were really assigned to the state laboratories and the state offices. And then they kept a cadre [of EIS officers] in Atlanta, who were sent out from Atlanta and who worked on the *MMWR* [*Morbidity and Mortality Weekly Report*], and who did other things there. But I think most of the EIS officers were sent out to outside assignments. This [work that I did] just was one of those [assignments].

LW: [I see, which caused you] to be involved in NIH evaluation. Well, I had thought that this would be about the point where we'd get to today [in our conversation], and [I'm please that] I've heard a lot about the hepatitis work. I'm wondering if we should stop for today, and I'll ask further questions if we meet a second time, if that would be okay with you.

RP: Sure, we can do that. That'll be fine. Why don't we just bring it kind of full circle around to the other two [hepatitis] viruses. You know, hepatitis has been a bigger problem in other countries than in the United States, so a lot of the people we've had in as postdocs [in the Hepatitis Viruses Section at LID] over the years have been from other countries where hepatitis is a *major* problem – Asia, not so many from Africa, but Europe particularly: Italy, Germany, France. We've had people from all over that part [of the world]. One individual from Italy had applied to come here. He was a young gastroenterologist, and he had just discovered a new antigen by immunofluorescence, looking in liver biopsies. This is [Dr.] Mario Rizzetto, and he called this thing "delta antigen." For reasons that escape me right now, we brought him over, and he worked principally in John Gerin's lab [at Georgetown University]. He was actually in an Expert Scientist position, I think, that I'd gotten for him from NIH. And that work went very well, because John's a very good scientist and knew what to do. And that then developed into the recognition that delta antigen was part of a transmissible agent, defective agent, [that] could be transmitted to chimps. And one of the interesting spin-offs is that hepatitis B vaccine protects against hepatitis D virus also, because if you don't have hepatitis B growing, you don't have hepatitis D growing. So that's three of the five viruses with a vaccine. We're still struggling with hepatitis C, but hepatitis E – I just want to tell you this because the public announcement of the field trial for the hepatitis E vaccine that we developed will be at the [annual meeting of the American Society of Tropical Medicine and Hygiene] next Monday in Washington. And so that's going to be very exciting, because it turns out, it's a very, very good vaccine.

LW: I didn't realize this.

RP: The problem is whether anybody will market it. It's licensed to GlaxoSmithKline, also, but it's a virus that is a major problem in developing countries, but not a problem in developed countries. So the market issue comes up. So we're hoping to figure out a way that it can be marketed, perhaps in Asia, under contract.

So that's been an interest of ours for several years. We're looking to see whether we can find another virus. So far we have not. There is evidence, there's epidemiological evidence probably [of] an additional [hepatitis] virus, particularly in developing countries, but we haven't been able to identify it yet.

LW: But you are doing some of that – I mean, do you take in samples then from places where you're saying the epidemiological evidence is showing? Or [do] you work partnership with labs in those places?

RP: Our thought was: the only animal that is susceptible to all five human hepatitis viruses is chimpanzees. If you were to pick one that might be susceptible to another virus, it would be the chimp. So we've tried transmitting materials from, probably by now, 30 different samples from almost as many different cases and outbreaks around the world to chimps without really reproducible clinical evidence of infection. But something that we've done in the past several years has led me to reevaluate that, and that is studies that we've been doing in collaboration with [Dr.] Frank Chisari at the Scripps Institute in La Jolla, California. Frank is a very good immunologist/pathologist/ virologist. Really, his training is in pathology, but he's had a big interest in immunology. We've been working together – he had developed the first transgenic mouse model for hepatitis B, in which the mice synthesized the whole hepatitis B virus, and if you take serum from these mice and inoculate a chimpanzee, it causes hepatitis B. They make virus, but of course it's part of the mouse genome, so it's not exactly the same as an infection. So he's really interested in some aspects of the infection that you can't do in the mice. We've teamed up – we do chimp studies with him, and he's supplied some very sophisticated analyses. So we've worked together on this. We've done this with hepatitis C, also.

And a couple of years ago, he set up, and we participated in, a microarray analysis of these, because we take weekly biopsies from the chimps, and divide up the biopsy and some were snap-frozen. Originally we did that for immunofluorescence, but it turns out those pieces of biopsy can be tested by microarray, and you see exactly what's happening in the liver. And, because the chimp's genome is so similar to that of humans, you can use the chimps that are made for human analysis in chimp studies. It's a beautiful thing. You know, one of the big problems in studying hepatitis in patients is, you don't see them until they're sick. And, depending on the virus, they're sick anywhere from two weeks to six months after they were infected. So you miss what's going on early on, which is where the host's interaction with the virus actually occurs. In the chimp, you see all of that.

LW: Because you're monitoring, you've done the challenge. Yes, I see. I think, even though I've read some of the [articles] that you gave me, the problem for me is that I've been reading a lot more about different kinds of diseases [that have been studied at LID], and I'm still [in the process of] understanding some of the particular aspects of hepatitis. So that's interesting, okay.

RP: What's emerged in immunology in recent years is, there has been a lot of interest in the adaptive immune response for a number of years, the T-cell/B-cell response, antibody response. There's been growing interest in recent years in the innate immune response, which is itself very complex, and occurs before the adaptive immune response, and the two actually have interactions. But there are hundreds of genes involved in the innate and in the adaptive immune response: all of the cytokines, all of the T-cell/B-cell related markers, all of these sorts of things. And the microarray allows you to look basically at all of these at once.

So, we're using the Affymetrix chip that has the entire human genome on it, so we can see all of these. And the real problem is determining what you're looking at – it's so complex. And monitoring the data, and just containing the data – *huge* amounts of data. In an experiment, we may have anywhere from 15 to 40 liver biopsies, weekly liver biopsies. Each of these will be looking at 20 thousand genes, 30 thousand genes. So it's an enormous amount of data, so just how to manage that is a problem.

But we've seen big differences in the host's response to hepatitis B versus hepatitis C. And you could make the diagnosis between those two, just looking at the host's response. For instance, in hepatitis B, there is *no* innate immune response. There's just an adaptive immune response. In hepatitis C, there's a very *strong* innate immune response, but it doesn't control the virus. So, here you have viruses with two different mechanisms: one is a stealth virus, it isn't seen by the [inaudible], and the other one *ignores* the immune response, by and large. There probably is some effect, but it can't control the infection. So we're now looking at [how these responses play out with] other viruses, because we'd done hundreds of these experiments over the years. They're all in the freezer, so we're looking at hepatitis A, hepatitis E, hepatitis D, and we can do this. Sometimes more than one virus over time [is found] in the same chimpanzee, so the chimp serves as its own control. Or we can look at the same virus in different chimpanzees to see if they respond in different ways.

But the interesting thing from this is: many times in chimpanzee studies, there really isn't any clinical response, but you can detect the infection, you can measure the viremia, you can measure the liver enzyme elevations. There may not even be liver enzyme elevations. By standard means, you can see an antibody response; you can say, "Well, this animal was infected, even though I didn't see any enzyme elevations; there wasn't any hepatitis, really, but there was an infection." And in those cases, the microarray analysis shows you this strong host response in the absence of any illness that you can see otherwise. So, our thought was, "Okay, maybe this is happening when we try to transmit non-A-through-E hepatitis virus to the chimp. Maybe we're infecting, but we just haven't been smart enough to know that. So if we look by microarray, maybe we will see that up-regulation of innate immune response or adaptive immune response genes, without actually knowing what that virus is or having specific diagnostic tools." So, we've begun looking at that. So far, we haven't found anything unequivocally positive, but we're still looking, and we have lots of stuff to look at.

But I think, if it's possible to infect a chimp, if there is another agent, if it causes *any* kind of host response, we'll find it. So we'll work it out, [and] that's a big interest of mine right now.

LW: The last thing that's coming to my mind – the last question that I'd like to ask you – [concerns] technology. The change in technology is really striking over the course of your career, and I think when I was coming in to ask you about hepatitis, I was [under the impression] that immune electron microscopy methods were [the instrumental in] making the new work [and progress over the years] possible. But now, the more I've read, I realize it really was a serendipitous thing[, as hepatitis experts such as Harvey Alter have used the concept of serendipity to characterize the early coincidences in hepatitis research], where people's work kind of came together, [for instance, for] finding the surface antigen. I'm wondering what role technology really [did play], the change in technology. It's kind of a two-part question. What is your impression of the change [that occurred] over your career? And then, [how would you characterize the significance of changes in technology for] making [advancements in hepatitis research] possible?

RP: I think hypothesis-driven research is greatly overrated. What drives research is technology, and people like [Dr. Leroy] Lee Hood have said this. He said, "You know, it's not hypothesis..." – hypothesis-driven research picks up the crumbs. It is discovery-based research, which is technology-based research, which opens up whole new avenues for you. Then you can ask questions, but by then the really exciting things, the new approaches, already have been opened up. And, just in the time I've been doing this, IEM was a new technique, and it was *very* valuable. Look, it found Norwalk virus; it found hepatitis A virus; it [generated the] first proof that there was a third hepatitis virus. It's been *very* important. Then, this was also the time when radioimmunoassays came along. Brand new technology: we got in very early on radioimmunoassays. We developed ones for antibody to hepatitis B, and we developed ones so you could detect Australia antigen – very sensitive. And we developed them for hepatitis A, so we went beyond IEM. And these were very exciting, because we were the first to show with a sensitive radioimmunoassay to hepatitis B that a lot more people had antibody than anybody suspected, a lot more than had any clinical disease. And then the same was true for hepatitis A. So radioimmunoassay was very important, and that was replaced by ELISA [or enzyme-linked immunosorbent assay], but that was kind of an incremental step. It did the same thing, and it just got labs away from having to use radioisotopes. You could do it with radioimmunoassay.

And, let's see, what after that? Of course, the ultracentrifuge, which was relatively new, was important for a lot of this, developing the vaccines. And then molecular biology – cloning and sequencing – was the next very important step that opened up all kinds of new possibilities. PCR [or polymerase chain reaction]: probably the most important advance of the twentieth century, if you want to know the truth. Done by somebody who wouldn't get tenure in any medical school or university in the country.

LW: Really? I don't know anything about that.

RP: [Dr.] Kary Mullis – oh, he was a strange guy. He worked for a small biotech company in California. He was really a surfer. That was his main interest. And he just had this thought while he was driving someplace – I think it was surfing, if I'm not mistaken – about how to answer some question, but it wasn't at all what it turned out to be. And it just turned out to be an extraordinarily important thing. And he got \$10,000 from the company; that's all he ever got from it.

LW: Amazing.

RP: So, it's those kinds of things – that was very important. I think microarray would be in that category. Certainly the whole informatics thing now, beginning with microarray and genomics, and then proteomics, which is now a big thing, and this whole series of "omics" that are coming along. It's coming so rapidly, and it's very hard to keep up with.

LW: Yes. It's pretty amazing.

RP: Things like spectrometry – mass spectrometry – which has been around for a while, but it's blossomed within just the last few years, so that you can now take vanishingly small amounts of things and sequence them in this machine and figure out what you have. You couldn't do this at all a few years ago. [It's] just amazing. And this is important in proteomics now; it's one of the key tools in proteomics. And that's just opening up all kinds of possibilities.

LW: Right. So it's not just the technology; it's figuring out a good use for it and developing it with that use in mind.

RP: Yes, but without technology... – that's what's really driving things so rapidly. I mean, computer technology drives me crazy. It's going to drive me to an early retirement. It drives me crazy, because I'm just too old to master that, but I can appreciate what it does, even if I can't do it. I have a GS-11 technician who's doing the analyses for all of my microarray work. I couldn't begin to do it myself.

Footnotes

[1] Cf. Robert M. Chanock February 1, 2001 interview, and his discussion of Drs. Nina and Gene Braunwald, of the Clinical Center and the National Heart Institute, and their role in the LID prospective heart surgery patient study.

[2] See S. M. Feinstone, A. Z. Kapikian, R. H. Purcell, "Hepatitis A: Detection by Immune Electron Microscopy of a Virus-like Antigen Associated with Acute Illness," *Science* 182 (Dec. 7, 1973): 1026-1028.

[3] Dr. Maassab was Professor of Epidemiology at University of Michigan's School of Public Health when he performed the research on cold-adapted viruses discussed here.